Rejection under 35 U.S.C. §103

The Examiner rejected claims 1-3 as being unpatentable over Nishijo et al. or Kaufman et al. In view of Neumann et al. or Khanna et al. (all already of record).

The Examiner states that Nishijo et al. teach a method of releasing a ligand from a complex formed with an endogenous protein by using benzoic acid and Kaufman et al. teach a method of releasing an acidic ligand from a complex with endogenous proteins using an effective amount of a releasing agent. The Examiner states that one of the alternative releasing agents disclosed in Kaufman is benzoic acid.

The Examiner states that Neumann et al. teach the specific use of o-methoxybenzoic acid for releasing a ligand from a complex. Khanna et al. teach a method of releasing a ligand from a complex comprising contacting a sample with a releasing agent, a preferred releasing agent being o-methoxybenzoic acid.

The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace Nishijo's or Kaufman's generic benzoic acid releasing agent with Neumann's or Khanna's specific benzoic acid derivative to produce the method of the instant invention, with a reasonable expectation of success and the invention is prima facie obvious.

Applicants respectfully traverse this rejection.

Kaufman et al. teach an antibody reagent for use in a fluorescence polarization immunoassay of 11-nor-Δ 9-tetrahydrocannibol-9-carboxylic acid which comprises an antibody and a releasing agent. The assay is used to test for the drug of abuse, marijuana, in urine samples. There is no suggestion that this method could be used for other ligands. Further, Kaufman et al. teach that the preferred releasing agents used in the fluorescence polarization immunoassay are bis-ANS, ANS and LDS (lithium dodecyl sulfate). These compounds, in addition to salicylate, are in fact the only compounds actually

tested in the Kaufman reference. Kaufman merely refers to "benzoic acid" in only one place in the specification on page 5, line 7, and in the claims. Kaufman does not teach or suggest any particular substitutions for the benzoic acid.

The Examiner admits that Kaufman et al. do not teach the use of the specific benzoic acid species of the instant invention as a releasing agent.

In fact, in the present specification, the Applicants have demonstrated that the methoxybenzoic acid isomers were more useful than ANS and salicylic acid, preferred compounds of Kaufman, in assays using these compounds as releasing agents. For example, Applicants have shown that while ANS can function as a releasing agent, it contributes detrimentally to background absorbance and the reagent discolors when exposed to light. This effect was seen even at low levels of 0.25mM. Page 34, line 11-15. Higher concentrations of ANS resulted in an offscale absorbance reading, preventing the collection of data. In contrast methoxybenzoic acid had little or no effect on background absorbance. See page 31-page 34, especially page 33, lines 13-16 and page 34, lines 8-22. Similarly, salicylic acid contributed 0.411 absorbance units above the control background at 340 nm and the salicylic acid preparation had a slight pink tinge. Because of these results, Applicants even eliminated salicylic acid from further comparative studies. See the specification, pages 34-35. Thus, the Applicants have clearly shown and disclosed in the present specification that the methoxybenzoic acids are unexpectedly much more useful as releasing agents in assays than the ANS or salicylic acid taught by Kaufman.

Applicants also respectfully disagree with the Examiner's interpretation of Nishijo. The Examiner cites Nishijo et al. as teaching a method of releasing a ligand from a complex formed with an endogenous protein such as serum albumin by using benzoic acid. The Examiner argues that Nishijo teaches that benzoic acid releases the ligand by competitive interaction with serum albumin.

Nishijo, in fact, merely provides a discussion of fundamental research into the thermodynamic properties of the binding of benzoic acid and theophylline and BSA, with no apparent or suggested practical application. Benzoic acid is used to elucidate and characterize theophylline binding to BSA. The thrust of Nishijo is to understand the nature and properties of BSA binding sites. For example, in the last sentence of the abstract, the authors conclude that "On the basis of all the experimental results, it is considered that theophylline and benzoic acid bind primarily to tryptophan residue on BSA by stacking due to van der Waals forces and secondarily bind to other amino acid residues on BSA thorough ionic and hydrophobic interactions."

One of ordinary skill in the art would not be motivated by the teaching of Nishijo to use benzoic acid, let alone a substituted benzoic acid, to release a ligand from a complex with endogenous proteins as presently claimed. One of ordinary skill in the art would need some other motivation to use the information provided in Nishijo for any practical purpose. One of ordinary skill in the art would simply not be motivated from Nishijo to use the compound recited in the claims to release a ligand from a complex with an endogenous protein by contacting a sample suspected of containing the complex with the compound as presently claimed.

The Examiner admits that Nishijo et al. do not teach the use of the specific benzoic acid species of the instant invention as a releasing agent.

The Examiner cites Khanna et al. and Neumann et al. as both teaching the use of methoxybenzoic acid as a releasing agent to release a ligand from a complex. However, as set for the in Applicants' previous response, there is simply no teaching in Khanna of the present invention, which is a **one step** method for releasing ligand from a complex with endogenous protein. Instead and to the contrary, Khanna uses a **three-step** method to prepare a concentrated sample of digoxin from a bodily fluid sample. First, Khanna discloses adding either a soluble or insoluble beta- cyclodextrin to the sample to bind digoxin, next the digoxin-(beta-cyclodextrin) complex is separated from the

sample (for a liquid beta-cyclodextrin using ultrafiltration techniques), next the beta-cyclodextrin is treated with a releasing agent to release the digoxin by contacting the complex with a releasing agent that can displace the digoxin by binding to the beta-cyclodextrin and then the amount of digoxin may be measured. The releasing agent may be, for example, p-methoxybenzoic acid. See col. 3, line 57.

In Khanna et al. cyclodextrin is added to the medium to bind digoxin. In the present invention, a methoxybenzoic acid compound is added to the medium to release a ligand from an endogenous protein. In contrast, Khanna specifically did not use a methoxybenzoic acid compound to bind digoxin in the medium or to release digoxin from endogenous proteins. Instead, Khanna used cyclodextrin. Thus, Khanna teaches that one needs to separate out digoxin first using cyclodextrin, then release the digoxin.

There is no motivation to combine Khanna with Kaufman or Nishijo because neither teaches an assay that uses cylcodextrin or any added component that forms a complex to be separated out prior to adding the releasing agent. Thus, there is simply no motivation to combine Khanna with Kuafman or Nishijo to arrive at the methods of the present invention. Furthermore, even if one of ordinary skill in the art were to combine the references, they would not arrive at the present invention. At most, they would arrive at a three step assay which (1) adds beta cyclodextrin to bind a ligand (assuming it would bind to the ligand), (2) separates the beta –cyclodextrin-bound ligand out from other components and (3) then adds a releasing agent to release the beta cylcodextrin from the ligand. The presently claimed one step method would clearly not have been obvious from these teachings.

Nor does Neumann supply the necessary motivation. The Neumann et al. invention relates to photography, and more particularly to photographic assemblages for color diffusion transfer photograph employing at least one silver

halide emulsion layer and a metallizable, redox dye-releaser (RDR). A certain ligand is employed in the processing composition. See, col. 1, lines 4-9.

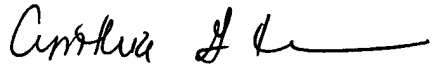
Metallizable redox RDRs are used in image transfer systems because of their exceptional stability to light. In the image transfer system, dye must be released rapidly from the RDR and must migrate rapidly to the mordant. Metallization and retention on the mordant must be efficient. Metallization occurs at a high pH (>12). See col. 1, lines 32-51. At this pH, many metals form insoluble hydroxides thus reducing the concentration of free metal ions available for dye metallization. This results in slow metallization rates. This invention provides a ligand that competes with hydroxide for the metal. See col. 1, lines 52-65. The ligands are disclosed at col. 2, lines 21-40. None of the ligands at col. 2, lines 21-40 are the releasing agents of the present invention. See also col. 2, line 55 to col. 3, line 15. None of the ligands listed are the releasing agents of the present invention.

The Examiner specifically points to Table V and col. 5 last paragraph. Applicants are unclear about the reference to col. 5, last paragraph as ligands or releasing agents are not discussed. It is noted that in Table V, o-methoxybenzoic acid is listed as a ligand under the heading "Comparison" as opposed to ligands of the Neumann invention which are listed under the heading "Invention". Table V also lists a control which has no ligand included. Col. 17, lines 25-30 states "the comparison compounds in this Table, which in many instances strongly hinder dye metallization, are informative in showing the specificity of this invention." Thus, Neumann teaches that o-methoxybenzoic acid does not work in the Neumann invention. Applicants ask that the Examiner compare the results in Table V for o-methoxybenzoic acid with the control and with the claimed compounds. O-methoybenzoic acid perfomed the same as or even worse than the control experiment (no compound) whereas the compounds under "Invention" performed better than the control. Thus, Applicants assert that one skilled in the art would not look to Neumann and elect to try methoxybenzoic acid. Instead, Neumann teaches the exact opposite and teaches away from the present invention. Methoyxbenzoic acid does not work in the Neumann

invention, thus there would be no motivation to combine Neumann with Kaufman or Nishijo. Instead, one skilled in the art would look at Neumann and decide not to try the comparison compounds including o-methoxybenzoic acid.

For all of the foregoing reasons Applicants respectfully requests that the rejection of the Examiner be withdrawn and that allowance of the claims be granted. If the Examiner believes that an interview would help to clarify any issue, the Applicants respectfully invite the Examiner to contact Applicants' attorney at the phone number listed below.

Respectfully submitted,



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